REMARKS

Applicants have carefully considered the points raised in the Office Action and believe that the Examiner's concerns have been addressed as described herein, thereby placing this case into condition for allowance, which is respectfully requested.

Claims 1-30 are pending and are currently under consideration. No claim amendments or cancellations have been made herein.

The specification has been amended to delete statements that hybridomas are being prepared for deposit with the ATCC. No new matter has been added.

Telephone interview

Applicants appreciate Examiners Cheu and Saunders extending the courtesy of a telephone interview on October 18, 2005.

An Interview Summary was faxed to Applicants' representative by Examiner Cheu. The Interview Summary states that the substance of the interview must be included in the response to the Office Action. In compliance with this requirement, Applicants submit that the pending claims and the present rejection under 35 U.S.C. §112, first paragraph, were discussed. The Examiners stated that this rejection would be withdrawn and that a further search of the art would be conducted.

Hybridoma deposit

The Examiner notes that the specification states that hybridomas designated 2.03, 2.04, and 2.11 are being prepared for deposit with the ATCC. The Examiner states that amendment of the specification to disclose the date of the deposit and complete name and address of the depository is required. By virtue of this response, the specification has been amended to delete the statement that

the hybridomas are being prepared for deposit, thus rendering the need to disclose the name and address of the depository moot.

Claim rejection under 35 U.S.C. §112, first paragraph

Claims 1-30 are rejected under 35 U.S.C. 112, first paragraph, as allegedly failing to comply with the enablement requirement. Applicants respectfully traverse this rejection.

The Examiner states that "[i]n view of . . . lack of predictability in the art, undue experimentation would be required to practice the claimed methods with a reasonable expectation of success, absent a specific and detailed description in the applicant's specification of how to effectively practice the recited method and absent working examples." Office Action, page 4. As an initial matter, Applicants respectfully note that "reasonable expectation of success" is the standard for obviousness, not enablement. (See, *e.g.*, MPEP §2143.02.) Therefore, the Examiner has applied an incorrect legal standard in making this rejection. The test for enablement is whether the specification teaches one of skill in the art how to make and use the claimed invention without undue experimentation. (See, *e.g.*, MPEP §1264.01.) Applicants respectfully submit that the claimed invention is enabled by the specification, as described below.

In the interview of October 18, 2005, and in the Interview Summary provided after the interview, the Examiner stated that the enablement rejection under 35 U.S.C. §112, first paragraph, would be withdrawn in view of Applicants' arguments that the specification teaches how to make and use the claimed invention. For completeness, Applicants' arguments in this regard are set forth below.

The specification teaches *how to make* the claimed digital antibodies. As discussed in detail on pages 31-40 of the specification, digital antibodies may be generated and characterized using techniques that are well known in the art. In Table 2, examples from the scientific literature of antibodies that bind small linear peptide epitopes are provided, demonstrating that it is possible to produce antibodies that bind epitopes of 3-5 amino acids.

Although working examples are not required for enablement (MPEP §2164.02), the claimed invention is exemplified in the working example provided on pages 71-81 of the specification. Mice were immunized with the peptides depicted in Table 4 on page 72 of the specification. Mice that demonstrated the strongest immune responses were selected for hybridoma fusions. Epitopes bound by the antibodies produced by the resulting hybridomas were mapped as described on page 78 of the specification and in Figure 1, using the mapping peptides described in Table 6 on pages 73-74. Eight of the hybridomas produced antibodies specific for the 5 amino acid epitope PEDTG, one hybridoma produced an antibody specific for the 3 amino acid epitope DTG, and one hybridoma produced an antibody specific for the 4 amino acid epitope KTTN. Thus, the specification exemplifies how to make antibodies that bind an epitope consisting of 3, 4, or 5 amino acids as claimed. Standard techniques for monoclonal antibody preparation and epitope mapping were used. It would not require undue experimentation on the part of a person of skill in the art to produce antibodies such as those exemplified in the specification.

The specification also teaches *how to use* the claimed digital antibodies. For example, methods for generating protein binding profiles with the claimed digital antibodies and using such protein binding profiles are described on pages 40-47, and methods for determining presence, absence, or identity of a protein of interest with the claimed digital antibodies are described on pages 48-52. The techniques described are standard methodologies used by those of skill in the art. Thus, a skilled artisan would understand how to use the claimed antibodies from the description provided in the specification.

On page 4 of the Office Action, the Examiner refers to a reference by Geysen et al. (1988) *J. Mol. Recognition* 1:32. The Examiner states that this reference teaches that amino acid replaceability within the epitope of an antigen that is recognized by an antibody is a "common problem" and would cause problems with regard to antibody specificity and recognition of proteins. The Examiner also states that Geysen et al. teach that on average about 4-5 amino acid residues are required to provide binding energy and specificity of antigen recognition and that smaller epitopes, such as 3 amino acids as claimed, would have a greater chance for replaceability and diminished specificity. Applicants respectfully disagree that this reference is applicable to enablement of the invention claimed in the present application. Replaceability of amino acids in epitope recognition

differs between antibodies and is dependent on the particular antibody being assessed. This point is illustrated in the following table which is extracted from the data presented in Geysen et al.

Parent amino acid	Replacement amino acid			
	D	E	S	T
D	100	50		
E	42	100		
S			100	22
T			44	100

Replaceability is not absolute and is much less than 100% for all of the amino acids shown in this table. For example, it is well known that aspartate (D) and glutamate (E) share similar physico-chemical properties, because both amino acids contain side chains that are acidic and negatively charged at neutral pH. However, in Geysen et al., when E was replaced by D, in 42% of the antibodies studied, binding to antigen was not significantly affected, while in 58% of the antibodies replacement of E by D affected binding of the antibody to antigen. Thus, a majority of the antibodies studied required residue E specifically and binding was significantly diminished when E was substituted with D. Therefore, it is possible to develop antibodies that are highly specific for the amino acid residues of a particular small epitope. This is taught in the specification in the working example discussed above, and in Geysen et al.

In the context of the claimed invention, antibodies that are specific for a small epitope of interest would be retained as a result of the screening procedure used in obtaining the antibodies (see Example 1) and antibodies that do not have the required specificity could be discarded. With regard to the Examiner's statement that epitopes containing 3 amino acids would be more subject to replaceability than longer epitopes, Applicants note that antibodies recognizing 3 amino acid epitopes are known in the art (see Table 2 of the specification) and are further enabled in this application by the antibody exemplified in Example 1 that specifically recognizes the epitope DTG.

As further support of the concept that it is possible to develop antibodies that specifically recognize a particular amino acid, and that are non-replaceable with respect to this amino acid, attached to this response as Exhibit 1 is a product data sheet from Chemicon International for anti-aspartate antibodies that do not show cross-reactivity with glutamate. Rabbit anti-aspartate

polyclonal antibody (Catalog no. AB132) cross-reacts with glutamate at 1/30,000 or 0.03%. Also attached as Exhibit 2 are product data sheets from Sigma-Aldrich for antibodies that are able to specifically distinguish between phosphorylated serine and threonine. A monoclonal antibody to phosphorylated threonine (Catalog no. P6623) does not react with phosphorylated serine and a monoclonal antibody to phosphorylated serine (Catalog no. P3430) does not react with phosphorylated threonine. The difference between the two amino acids is in the side chain and these antibodies are able to distinguish the difference. Further, a product data sheet is attached from Chemicon International as Exhibit 3 for an antibody that specifically distinguishes between D-aspartate and L-aspartate. The cross-reactivity of the anti-D-asparate rabbit polyclonal antibodies (Catalog no. AB5769) with L-aspartate is less than 1/10,000 and the cross-reactivity with D- or L-glutamate is less than 1/50,000. Thus, it is possible to develop antibodies with very fine specificity that are capable of distinguishing between different amino acids and for which the amino acid recognized is not replaceable.

As discussed above, the Examiner stated both in the telephone interview of October 18, 2005 and in the Interview Summary that the rejection under 35 U.S.C. §112, first paragraph, would be withdrawn. Applicants would appreciate the Office officially withdrawing this rejection.

Withdrawal of previous rejections

Applicants acknowledge with appreciation the withdrawal of the previous rejections under 35 U.S.C. §§ 102(b), 102(e), and 103, to the extent that they are not reiterated. Applicants would appreciate the Office officially withdrawing these rejections.

CONCLUSION

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, Applicants petition for any required relief including extensions of time and authorize the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 03-1952 referencing docket no. 559312000100. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: October 20, 2005

Respectfully submitted,

By jill a. jacobson

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RABBIT ANTI-ASPARTATE POLYCLONAL ANTIBODY

CATALOG NUMBER: AB132

LOT NUMBER:

QUANTITY: 100 µL, sufficient for approximately 75-100 immunohistochemical determinations.

SPECIFICITY: Aspartate (L-Aspartate, L-Aspartic acid)

The cross-reactivities were determined using either ELISA or RIA techniques, at concentration/unconjugated or conjugated amino acid concentration at half displacement.

Compound	Cross-reactivity
L-Aspartic acid-G-BSA	1
L-Glutamic-G-BSA	1/30,000
GABA-G-BSA	1/>100,000

The antisera was also tested for specificity using the free-floating PAP technique on rat cortex.

Abbreviations:

(G) Glutaraldehyde
(BSA) Bovine Serum Albumin

IMMUNOGEN: L-Aspartate-gluteraldehyde-BSA

APPLICATIONS: Immunohistochemistry: 1:1,000-1:2,500 by PAP (see suggested protocol). Optimal working dilutions must be determined by the end user.

FORMAT: Rabbit antiserum.

PRESENTATION: Liquid with 0.05% sodium azide.

STORAGE: Maintain at -20°C in undiluted aliquots for up to 12 months after date of receipt. Avoid repeated freeze/thaw cycles.

REFERENCES:

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SAMPLE PROTOCOL for Neurotransmitter Detection by Immunocytochemistry. Example for a rat brain.

1. **SOLUTIONS TO BE PREPARED** - Solution must be prepared as needed.
Note:Tris can be replaced by a 0.01M phosphate solution.

Solution A: 0.1 M cacodylate acid, 10 g/L sodium metabisulfite, pH 6.2.(*)

Solution B: 0.1 M cacodylate acid, 2.5-5% glutaraldehyde, 10 g/L sodium metabisulfite, pH 7.5.(*)

Solution C: 0.05 M Tris, 8.5 g/L sodium metabisulfite, pH 7.5.(*)

Solution D: 0.05 M Tris, 8.5 g/L sodium chloride pH 7.5.(*)

(*) Adjust pH with NaOH or HCl if necessary.

In the case of **GLUTAMATE**, Tris can be replaced by .01 M PBS in solutions C and D.

2. **RAT ANAESTHESIA** - The rat is anaesthetized with sodium pentobarbital or chloral hydrate. The anaesthesia is correct when: on its' back, rat doesn't return to it's side & light reaction occurs pinching the tail.

3. **RAT PERFUSION** - Open the animal's thorax and rapidly cannulate the aorta via the left ventricle. Cut the right atrium or ventricle to allow efflux of blood and perfusate. Clamp off the descending aorta. Perfuse intracardially through the aorta, using either a multi-speed pump or a large syringe.

Solution A (30 mL):	150-300mL/mn
Solution B (500 mL):	150 mL/mn

Solutions A and B must be perfused through the rat brain continuously without flow stopping when changing solutions.

<u>Indications of a good perfusion:</u>
- -Limbs are blanching. Ears are bleached and very white.
- -Liver loses it's color and becomes very hard.
- -When cutting the rat nose, glutaraldehyde must leak drop by drop.
- -The brain must be dark-yellow and hard. (The color is homogeneous without any white blots).

<u>Indications of an incorrect perfusion:</u>
- -All the above indications do not appear.
- -Glutaraldehyde leaks by the mouth. Rat eyes are swollen.

4. **POST FIXATION:** Cover rat brain with Solution B and let soak 30-120 minutes, then soft wash 4 times in Solution C.

5. **TISSUE SECTIONING:** 50 um slices, preferably by the "vibratome" technique, using Solution C.

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PROTOCOL (cont)

6. **WASHING:** The sections are washed 3X in cold (4 deg) Sol'n C, then incubated 1-1.5 hrs at room temp. in Sol'n C plus 3% of non-specific serum (normal goat serum).

7. **PRIMARY ANTIBODY:** Use a final dilution of 1:200-1:500 in Solution C containing 0.2% Triton X100 and 1% non-specific serum. Incubate 12 sections per 2 mL diluted antibody overnight, +4°C. Then wash the sections three times for 10 minutes each in Solution D. (Note that the antibody may be usable at a higher dilution. This should be explored to minimize the possibility of high background. Additionally, note that a change in the buffering system as indicated in the protocol may change the background and antibody recognition). The specific reaction is then revealed by PAP procedure.

8. **SECOND ANTIBODY:** Incubate the sections with a 1:50 to 1:200 dilution of goat anti-rabbit in Solution D containing 1% non-specific serum for either 3 hrs at 20°C or 2 hr at 37°C. Then wash the sections, 3 times, for 10 minutes each with Solution D.

9. **PAP:** Incubate the sections with the appropriate dilution of peroxidase anti-peroxidase (for free floating method) in Solution D containing 1% non-specific serum for 1-2 hours at 37°C. Then wash sections 3 times for 10 min each in solution D.

10. **VISUALIZATION:** The antigen-antibody complexes are visualized using DAB-4-HCl (25 mg/100 mL) in 0.05M Tris and filtrated; 0.05% hydrogen peroxide is added. Incubate the sections for 10 minutes at room temp. Stop the reaction by transferring the sections to 5 mL 0.05M Tris. Wash tissue with solution D using 2, 10 min washes. Mount sections on chrome-alum coated slides. Dry overnite at 37°C. Rehydrate sections using conventional histological procedures. Coverslip using rapid mounting media.

For research use only; not for use as a diagnostic.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µl or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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ProductInformation

MONOCLONAL ANTI-PHOSPHOSERINE
CLONE PSR-45
Mouse Ascites Fluid

Product Number **P 3430**

Product Description

Monoclonal Anti-Phosphoserine (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. Phosphoserine conjugated to KLH was used as the immunogen. The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Phosphoserine reacts against phosphorylated serine both as free amino acid or when conjugated to carriers such as BSA or KLH using ELISA and dot blot. It does not react with non-phosphorylated serine, phosphorylated tyrosine or threonine, AMP, or ATP. The antibody has been used for the localization of some phosphoserine containing proteins using the immunoblotting method. Certain proteins known to contain phosphorylated serine may not be recognized by this antibody because of steric hindrance of the recognition site.

Protein phosphorylation and dephosphorylation are basic mechanisms for the modification of protein function in eukaryotic cells.[1] Phosphorylation is a rare post-translational event in normal tissue, however, the abundance of phosphorylated cellular proteins increases tenfold following various activation processes which are mediated through phosphotyrosine, phosphoserine, or phosphothreonine (p-tyr/p-ser/p-thr). Many different mitogenic systems, such as the EGF, PDGF, and insulin receptor systems contain tyr/ser/thr kinase domains which autophosphorylate specific tyr/ser/thr residues upon binding of their ligands.[2] T cell antigen receptor complex or the receptors for some hemopoietic growth factors may stimulate associated kinases,[3] and cells transformed by viral oncogenes contain elevated levels of phosphorylated tyr/ser/thr. An understanding of transformation by oncogenes and mitogenic processes of growth factors depends on the identification of their substrate and a subsequent determination of how phosphorylation affects the properties of these proteins. Studies on the role of phosphorylated proteins have been hampered by their low abundance and the problem of distinguishing the various types of phosphorylated proteins. The most common procedure is to label intact cells or small tissue fragments with ^{32}P and subsequently to isolate ^{32}P-labeled proteins by conventional biochemical methods. In order to identify the specific amino acids that undergo phosphorylation, additional long and tedious procedures for phosphoamino acid analysis are required. Immunoblotting of cellular proteins with antibodies directed against phosphoamino acids is advantageous as it does not involve ^{32}P labeling, and can therefore be employed to monitor alterations in phosphorylation of specific proteins as they occur in intact organs or even whole animals. Indeed, mono- and polyclonal antibodies directed against phosphorylated residues were generated and found useful as analytical and preparative tools[2,4] by enabling the identification, quantification and immunoaffinity isolation of phosphorylated cellular proteins.

Reagents

The product is provided as ascites fluid with 15 mM sodium azide as a preservative.

Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, solution may be frozen in working aliquots. Repeated freezing and thawing is **not** recommended. If slight turbidity occurs upon prolonged storage, clarify by centrifugation before use.

Product Profile

1. A minimum working dilution of 1:4,000 is determined by indirect ELISA using microtiter plates coated with phosphoserine conjugated to BSA (10 µg/ml).
2. A minimum working dilution of 1:500 is determined by indirect immunoblotting using an extract of rat brain cortex.

In order to obtain optimum results it is recommended that each individual user determine their optimum working dilutions by titration assay.

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JWM/daa 7/02

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ProductInformation

Monoclonal Anti-Phosphothreonine
Clone PTR-8
Purified Mouse Immunoglobulin

Product Code **P 6623**

Product Description

Monoclonal Anti-Phosphothreonine (mouse IgG2b isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells (NS 1) and splenocytes from BALB/c mice immunized with phosphothreonine conjugated to KLH. The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Phosphothreonine reacts with phosphorylated threonine both as a free amino acid or when conjugated to carriers such as BSA or KLH, using ELISA and dot blot. It does not react with non-phosphorylated threonine, phosphorylated tyrosine or serine, AMP or ATP.

The antibody may be used for the detection of phosphorylated threonine using various immunochemical assays such as ELISA, dot blot,[1] immunoprecipitation,[2] immunocytochemistry[2] and immunoblotting.[3-5] Due to steric hindrance of the recognition site, this antibody may not recognize certain proteins known to contain phosphorylated threonine.

Protein phosphorylation and dephosphorylation are basic mechanisms for the modification of protein function in eukaryotic cells.[6] Phosphorylation is a rare post-translational event in normal tissue. However, the abundance of phosphorylated cellular proteins increases tenfold following various activation processes, which are mediated through phosphotyrosine, phosphoserine or phosphothreonine (p-Tyr/p-Ser/p-Thr). Many different mitogenic systems, such as the EGF, PDGF and insulin receptor systems, contain Tyr/Ser/Thr kinase domains that autophosphorylate specific Tyr/Ser/Thr residues upon binding of their ligands.[7] T cell antigen receptor complex or receptors for some hemopoietic growth factors may stimulate associated kinases,[8] and cells transformed by viral oncogenes contain elevated levels of phosphorylated Tyr/Ser/Thr. An understanding of transformation by oncogenes and mitogenic processes of growth factors depends on the identification of their substrate and a subsequent determination of how phosphorylation affects the properties of these proteins. Immunoblotting of cellular proteins with antibodies directed against phosphoamino acids has been the method of choice for studying the role of certain phosphorylation events. Antibodies can be employed to monitor alterations in phosphorylation of specific proteins as they occur in intact organs or even whole animals. Indeed, mono- and polyclonal antibodies directed against phosphorylated residues were generated and found useful as analytical and preparative tools[9],b y enabling the identification, quantification and immunoaffinity isolation of phosphorylated cellular proteins.

Reagent

The antibody is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Antibody Concentration: approx. 2.5 mg/ml.

Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze at –20 °C in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile
A working concentration of 5-10 µg/ml is determined by immunoblotting, using A431 cell extracts.

A working concentration of 0.5-1.0 µg/ml is determined by indirect ELISA using phosphothreonine conjugated to BSA (Product Code P 3842, 10 µg/ml) as the coating substrate.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

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EK,MCT,PHC 05/05-1

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RABBIT ANTI-D-ASPARTATE (D-ASPARTIC ACID) POLYCLONAL ANTIBODY

CATALOG NUMBER: AB5769

LOT NUMBER:

QUANTITY: 100 µL

SPECIFICITY: D-Aspartate (D-Aspartic acid)

The cross-reactivities were determined using the ELISA technique by competition experiments:

Compound	Cross-reactivity
D-Aspartate-G-BSA	1
L-Aspartate-G-BSA	1/>10,000
D-Glutamic-G-BSA	1/>50,000
L-Glutamic-G-BSA	1/>50,000
N-Methyl-D-Aspartate	1/>50,000

Abbreviations:
(G) Glutaraldehyde
(BSA) Bovine Serum Albumin

IMMUNOGEN: D-Aspartate-gluteraldehyde-BSA

APPLICATIONS: Immunohistochemistry: 1:500-1:2,500
ELISA: 1:500-1:2,500
Optimal working dilutions must be determined by the end user.

SPECIES REACTIVITY: Rat. Other species have not been tested.

FORMAT: Rabbit serum.

PRESENTATION: Liquid with 0.05% sodium azide.

STORAGE/HANDLING: Maintain at -20°C in undiluted aliquots for up to 6 months after date of receipt. Avoid repeated freeze/thaw cycles. The antibody may be diluted (1:1) in glycerol (ACS grade or better) for greater stability.

For research use only; not for use as a diagnostic.

Important Note: *During shipment, small volumes of antibody will occasionally become entrapped in the seal of the product vial. For antibodies with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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PROTOCOL for Detection by Immunohistochemistry. Example for a rat brain.

RAT PERFUSION:

The rat is anaesthetized with urethane (0.5-1.5g/kg; intraperitoneal). Heparinized, and perfused via the ascending aorta with 100 mL of cold physiological saline (0.9% NaCl) and with the following fixative solution:

a) 300 mL of cold 4% paraformaldehyde with 2% glutaraldehyde in 0.1M phosphate-buffer (PB), pH 7.2 (2 minutes).
b) 600 mL of cold 4% paraformaldehyde with 2% glutaraldehyde in 0.1M phosphate-buffer (PB), pH 7.2 (10 minutes).
c) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M phosphate-buffer (PB), pH 7.2, at 4°C for 12 to 16 hours.
d) Before the brains will be cut on a freezing microtome, you must include the brain in increasing concentrations of sucrose (5% sucrose in PBS until the brain sinks) after that repeat the same process in a solution with a higher level of sucrose 10%, 20%, 25% and finally 30%. Sections of ~ 50 μm thickness should be obtained and kept at 2-8°C in PBS (0.1M, pH 7.2) and processed for immunostaining.

APPLICATION OF D-ASPARTATE ANTIBODY:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections should be treated with distilled water containing NH_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H_2O_2 and 66% of methanol).
2. Wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2).
3. Pre-incubate the sections for 30 min in PBS containing 10% of normal serum from the host of your secondary antibody and 0.3% of Triton X-100 (blocking solution).
4. Incubate at room temperature (1 hour 30 min) and then overnight at 2-8°C with AB5769 appropriately diluted in blocking solution (optimal dilutions must be determined by the end user).
5. Wash sections in PBS for 30 min.
6. Incubate for 60 min at room temperature with biotinylated anti-rabbit IgG (for example Chemicon Catalog number AP132B) diluted appropriately in PBS.
7. Wash sections for 30 min with PBS.
8. Incubated sections for 1 hour with appropriately diluted avidin/streptavidin-peroxidase complex.
9. Wash the sections in PBS (30 min).
10. Wash with Tris-HCl buffer (pH 7.6) (10 min).
11. The tissue-bound peroxidase should be developed with H_2O_2 using 3, 3' diaminobenzidine as chromogen.
12. Finally rinse sections with PBS and cover slip with PBS/Glycerol (1:1).

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